Vision Research 51 (2011) 470–478

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Orientation tuning in the visual cortex of 3-month-old human infants

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ARTICLE INFO

Article history:
Received 19 April 2010
Received in revised form 30 December 2010
Available online 12 January 2011

Keywords:
Orientation tuning
Visually Evoked Potential
Receptive field
Non-classical surround

ABSTRACT

Sensitivity to orientation is critical for making a whole and complete picture of the world. We measured the orientation tuning of mechanisms in the visual cortex of typically developing 3-month-olds and adults using a nonlinear analysis of the two-input steady-state Visually Evoked Potential (VEP). Two gratings, one a fixed test and the other a variable orientation masker were tagged with distinct temporal frequencies and the corresponding evoked responses were measured at the harmonics of the test and masker frequencies and at a frequency equal to the sum of the two stimulus frequencies. The magnitude of the sum frequency component depended strongly on the relative orientation of the test and masker in both infants and adults. The VEP tuning bandwidths of the 3-month-olds measured at the sum frequency were similar to those of adults, suggesting that behavioral immaturities in functions such as orientation discrimination and contour integration may result from other immaturities in long-range lateral projections or feedback mechanisms.

1. Introduction

The ability to detect stimulus orientation is one of the critical first steps in making a cohesive picture of the visual world. Fortunately, the human visual system is well equipped for detecting and discriminating orientation (e.g. Campbell & Kulikowski, 1966; Regan & Beverly, 1985). For example, adults can discriminate less than a 1° difference in line orientation (Vàzquez, Cano, & Acuña, 2000; Westheimer, Shimamura, & McKee, 1976).

Studies of animal models have demonstrated that tuning to local orientation first emerges in V1 (Hubel & Wiesel, 1959, 1962, 1968; Schiller, Finlay, & Volman, 1976), and is largely absent in earlier stages of visual processing (e.g. the LGN; Shapley, Hawkin, & Xing, 2007). The tuning is thought to arise from a combination of feed-forward afferents and local cortical networks (e.g. Angelucci & Bressloff, 2006; Ferster & Miller, 2000; Shapley, Hawkin, & Ringach, 2003).

The best information about the orientation tuning of individual neurons relevant to human vision is derived from single cell recordings from macaque monkeys. Hubel and Wiesel (1968) were the first to describe how simple and complex cells respond to the orientation offset of lines. Orientation tuning half-width at half-height data from DeValois, Albrecht, and Thorell (1982) had a median value of around 42° in anesthetized monkeys but similar measurements in alert monkeys have been found to depend on cell type and cortical lamina (Gur, Kagan, & Snodderly, 2005). The narrowest tunings reported in the alert macaque are as low as 11.5° at half-width at half-height (Gur et al., 2005). A further factor contributing to orientation tuning estimates is the size of the stimulus – larger stimuli have been shown to yield narrower orientation-tuning bandwidths (Gur et al., 2005; Xing, Shapley, Hawken, & Ringach, 2005). There is thus a wide range of reported tuning bandwidths for individual neurons.

Human infants are capable of discriminating large differences in the orientation of lines a week after birth (e.g. 45° versus 135°) (Atkinson, Hood, Wattam-Bell, Anker, & Tricklebank, 1988; Slater, Morison, & Somers, 1988). Furthermore, Bornstein, Krinsky, and Benasich (1986) demonstrated that by 4-months infants can discriminate 10° differences in line orientation behaviorally and Manny (1992) suggested, using a VEP (Visually Evoked Potential) technique, that 3-month-olds may be able to discriminate changes in orientation of around a degree.

The basic development of orientation-tuned neurons does not require postnatal visual experience in some species. Wiesel and Hubel (1974) demonstrated this in newborn monkeys by suturing shut the eyes, and finding that, without visual input, the monkeys still possessed orientation-tuned columns that were adult-like (see also Crawford, Pesch, von Noorden, Harwerth, & Smith, 1991). There are human data suggesting that orientation-discrimination mechanisms are not in place until at least 2-months of age (Braddick, Wattam-Bell, & Atkinson, 1986), but Braddick (1993) in his review of the human development literature has suggested that the quality of orientation-specific behavioral or evoked potential responses over the first weeks after birth depends on the characteristics of the stimulus (temporal frequencies, spatial frequencies, etc.). Further, these authors (Braddick, 1993; Braddick

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doi:10.1016/j.visres.2011.01.003

et al., 1986) have suggested that different aspects of orientation processing (e.g. tuning of individual neurons or behavioral discrimination) might depend on the maturation of different aspects of cortical processing – excitatory, inhibitory, feed-forward or lateral circuitry for example. Candy, Skoczenski, and Norcia (2001) suggested that the different maturation rates of discrimination and masking performance were consistent with a normalization-type model of cortical processing, incorporating orientation-tuned units and a divisive normalization pool.

Given the potential neural complexity of orientation processing, postnatal immaturities in orientation processing should not be surprising. There is considerable neuronal maturation over the first few years of life (Johnson, 1990, 1997). Further, Burkhalter, Bernardo, and Charles (1993) have argued that horizontal connections in V1 are in place only at 4-months of age in human, and that although long-range projections in layer 2/3 are evident at 4-months of age, they do not become adult-like until 15-months postnatally.

Despite the aforementioned behavioral and VEP data collected from human infants, there are no studies that we are aware of that have examined the orientation tuning of mechanisms in the developing human visual cortex. Orientation tuning in humans must be estimated using non-invasive measures such as a VEP tuning bandwidth or behavioral thresholds. For adults, the half-widths at half-height estimated psychophysically are on the order of 12° (e.g. Campbell & Kulikowski, 1966) and for VEP studies of adult humans half-bandwidth half-heights of 6° (Regan & Regan, 1987) have been reported.

2. Experiment I

2.1. Methods

2.1.1. Participants

Twenty-nine infants (9 males; 20 females) participated. They ranged in age from 2.9 to 3.9 months ($M = 3.40$, $SD = 0.50$). Informed consent was obtained from their parents after the local Indiana University IRB had approved the study. Seven adults with corrected to normal vision also participated in this study. They ranged in age from 24 to 52 years ($M = 29.22$, $SD = 5.24$). Informed consent was also obtained from these adults.

2.1.2. Stimuli

Stimuli were generated using a Power Macintosh G4 computer running PowerDiva software (see Candy et al., 2001) and presented on a gamma-corrected monochrome monitor (800 × 600 pixels at a 72 Hz; Richardson Electronics MR-2000). Participants viewed two overlapping spatial sinusoids that were presented in interlaced frames at 40% Michelson contrast. They subtended 12° in a square, with a mean luminance of 104 cd/m^2 (as shown in Fig. 1). One of the sinusoids remained vertical (the test) and was counterphase-reversed at 3.27 Hz (f1). The second spatial sinusoid (the mask) was presented at orientation offsets relative to the test of 0°, 3°, 5°, 7°, 15°, 30°, and 90° and was counterphase-reversed at 5.14 Hz (f2). The spatial frequency of the sinusoids presented to the infants was 1 cpd. The adults were presented with three conditions, the 1 cpd sinusoids that were presented to the infants, plus an otherwise equivalent pair of 5 cpd sinusoids subtending 12°, and a pair of 5 cpd sinusoids subtending 2.7° (a factor of five smaller). These conditions presented to adults were designed to include various simple models of how receptive field structure might mature in humans. A developmental model of stable 2D receptive field structure and increasing sensitivity with maturation (as discussed by Banks and Crowell (1993)) would predict that the 1 cpd stimulus used with the infants would be the appropriate comparison to stimulate the equivalent mature neurons in adults. If the tuning of receptive fields were to shift to higher spatial frequencies with development (as modeled by Wilson (1988)), after photoreceptor migration results in increased photoreceptor density for example, a neuron tuned to one spatial frequency at birth would mature to be tuned to a higher spatial frequency in adulthood.

Taking the maturation of VEP acuity as a guide (in that it is representative of neurons tuned to the highest spatial frequencies across age (Norcia & Tyler, 1985; Wilson, 1988)) one might predict a factor of five change in spatial frequency selectivity of a neuron between 3-months of age and adulthood under this model. Adults were therefore tested on the 5 cpd conditions to stimulate the equivalent mature neurons under this model. The area of the 5 cpd stimulus was scaled down in the third condition by a factor of five, to be consistent with a full 2D receptive field-scaling model (scaling of both spatial frequency tuning and receptive field size, to maintain a constant number of cycles per receptive field across development). It is unlikely that any one of these models is

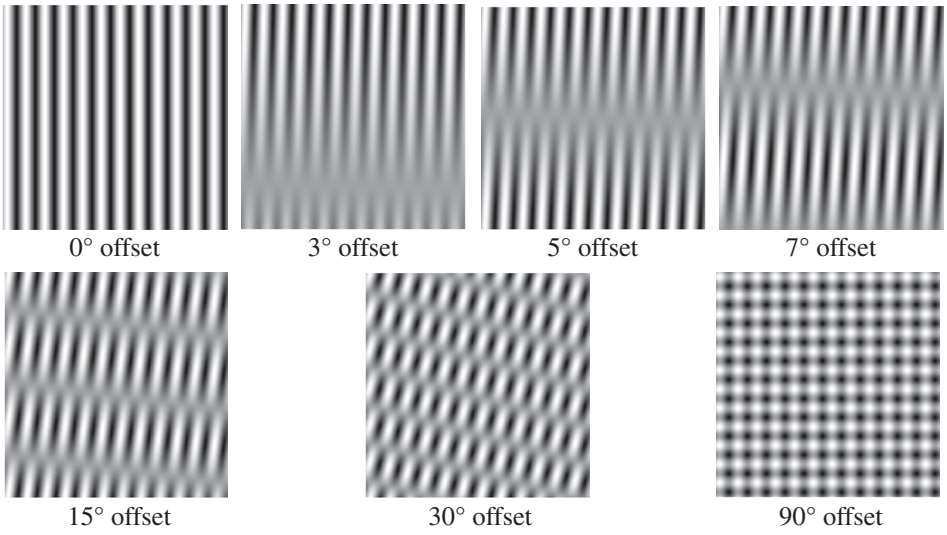

Fig. 1. Frames taken from the 1 cpd stimuli at different orientation offsets. The test stimulus (which counterphase-reversed at 3.14 Hz) and the mask stimulus (which reversed at 5.27 Hz) were superimposed in interlaced frames, with the test remaining vertical while the mask was rotated to the appropriate orientation offset. The number under each image indicates the mask offset in degrees.

appropriate for the entire retinal area stimulated by the 12° stimulus, as a result of the non-homogenous postnatal migration of photoreceptors for example, but, given the complexity of the real process, all three were included in an attempt to encompass the extreme possibilities.

2.1.3. EEG

Participants sat at 70 cm from the stimulus. Infants were seated on the lap of their caregiver. The stimuli were presented in a pseudo-randomized order with adults completing all orientation-offset conditions and infants completing as many as their attention span would allow, with the exception that all infants completed the baseline, aligned condition (0°), first.

The VEP was recorded in 21.4 s trials (3 per orientation condition for infants and 6 per orientation condition for adults). The EEG was collected with Grass gold-cup surface electrodes in a 5-electrode montage (0_1, 0_z, 0_2, with ground and reference at P_z and C_z, respectively). The response from the following two channels was analyzed: 0_1 referred to 0_z and 0_2 referred to 0_z with the channel with the most reliable signals across conditions, based on t^2 *circ*, included in the analysis. The electrode impedance was less than 20 kΩ. The EEG was bandpass filtered between 1 and 100 Hz and amplified by a factor of 50,000 for adults and 20,000 for infants, using a Grass Model 12D amplifier. It was then sampled at 433 Hz.

2.1.4. Analysis

A recursive least squares filter was used to calculate the average amplitude and phase spectra across trials for each condition and subject (Tang & Norcia, 1995). The t^2 *circ* (Victor & Mast, 1991) statistic was then used to determine the significance of the response at each frequency of interest. This statistic employs both the distribution of amplitudes and phases across trials. Analysis of Variance (ANOVA) and post-hoc tests were performed with SPSS (Version 11.0, SPSS: www.spss.com) and further statistical analyses were then performed using STATA (Version 11.0, STATA Corporation: www.stata.com/).

The theory behind the nonlinear analysis is as follows, if two stimuli modulating at different temporal frequencies are passed through a non-linearity such as a spike threshold, the non-linearity will generate distortion or intermodulation (IM) terms at specific additional temporal frequencies (second-order nonlinear terms could be generated at the arithmetic sum and difference of the two input temporal frequencies for example). If the non-linearity is orientation selective, the presence of the IM will depend on the relative orientation of the two stimulus inputs and the orientation bandwidth of the mechanism. Regan and Regan (1987) used this approach with adults and found a strong nonlinear response to two optically superimposed parallel gratings. The minimum of this response was at 30° of offset for their two subjects and their half-bandwidth at half-height was approximately 6°.

Counterphase-reversing stimuli typically generate a response at the second harmonic of the stimulus temporal frequency (Regan & Regan, 1988). In this study these responses were therefore expected at 2f1 (6.54 Hz) and 2f2 (10.28 Hz), and were classified as the 'self-terms'. The signature of processing by a common non-linearity was predicted to lie at the IM terms, (the second-order sum term (f1 + f2 or 8.41 Hz), in particular, as the second-order difference term (f2 − f1 or 1.87 Hz) was at too low a temporal frequency to result in a significant response).

2.2. Results

Data from 14 infant participants were discarded because their EEG amplitudes failed to reach a significant t^2 *circ* signal for the 2f1 self term in the baseline aligned, 0° offset, condition (n = 13) and one infant tested was too old to be included in the data set (>4.5 months). The 15 included infants ranged in age from 2.9 to 3.9 months (M = 3.34, SD = 0.30).

Each individual's amplitudes at 6.54 (2f1), 10.28 Hz (2f2), and 8.41 Hz (second-order sum intermodulation) were each normalized to the amplitude at that frequency for the baseline aligned condition. The second-order difference frequency (1.87 Hz) was excluded from these analyses because it did not reach significance for numerous participants. Not all infants completed each condition and the number of infants for each orientation offset is shown in Table 1. We required that there were at least seven infants for each orientation, and that they had completed the aligned condition to permit the normalization. The infants completed a mean of 4.7 out of 7 of the orientation offsets (range 2–7 offset conditions). The adults each completed the full set of orientation offsets for each of their three stimulus conditions (1 cpd, 5 cpd, and 5 cpd scaled).

2.2.1. Spectral analysis

We were first interested in the second harmonics of the test (2f1 = 6.28 Hz) and mask (2f2 = 10.58 Hz) frequencies, the self-terms (see Fig. 2). These terms would be expected to demonstrate the masking influence of one stimulus on the other, while retaining the specific temporal signature of the relevant stimulus. In other words, the response at 2f1 would indicate the masking effect of the mask on the test response and the response at 2f2 would indicate the effect of the test on the mask, without being contaminated at the electrode by the direct response to the other stimulus frequency. In the case of the self-terms, we might expect to find the amplitudes at 2f1 and 2f2 increasing with increasing orientation offsets, resulting from a release from masking (e.g. Morrone & Burr, 1986).

Therefore, to understand any differences across the orientation offsets and between the groups for the second harmonics of the test, a two-way ANOVA for the 2f1 frequency was performed with orientation offset (0°, 3°, 5°, 7°, 15°, 30°, 90°) as the within subjects factor and group (Infants 1 cpd, Adults 1 cpd, Adults 5 cpd, and Adults 5 cpd scaled) as the between subjects factor. See Table 2 for the main effects and post-hoc comparisons. The post-hoc tests with Bonferroni correction to the alpha showed that infants had significantly larger amplitude 2f1 responses, overall, compared to adults except for adults in the 5 cpd condition.

A corresponding two-way ANOVA for the 2f2 frequency was performed with orientation offset (0°, 3°, 5°, 7°, 15°, 30°, 90°) as the within subjects factor and group (Infants 1 cpd, Adults 1 cpd, Adults 5 cpd, and Adults 5 cpd scaled) as the between subjects factor. See Table 3 for the main effects and post-hoc comparisons.

In contrast to the 2f1 analysis above, the 2f2 response amplitudes were smaller for infants compared to adults in the 5 cpd scaled condition, and no different compared to adults in any other condition. These data are not included in Fig. 2 for the sake of clarity, but they demonstrate the same qualitative effect as the 2F1 data in that there are no main effects of offset or interaction.

The 2f1 and 2f2 data do not reveal any release from masking with increasing orientation offset. Although somewhat surprising, this result is consistent with the data of Candy et al. (2001), who looked at the effect of parallel and orthogonal masks on the amplitude of the self-terms. The effects of the maskers on the self-terms were not orientation specific (their Fig. 3) for adults or 3-month-olds at 40% contrast. These self-responses are also quite variable

Table 1
The number of infants that completed each orientation-offset condition.

Orientation offset (in °)	0	3	5	7	15	30	90
Infant n	15	10	8	10	7	7	8

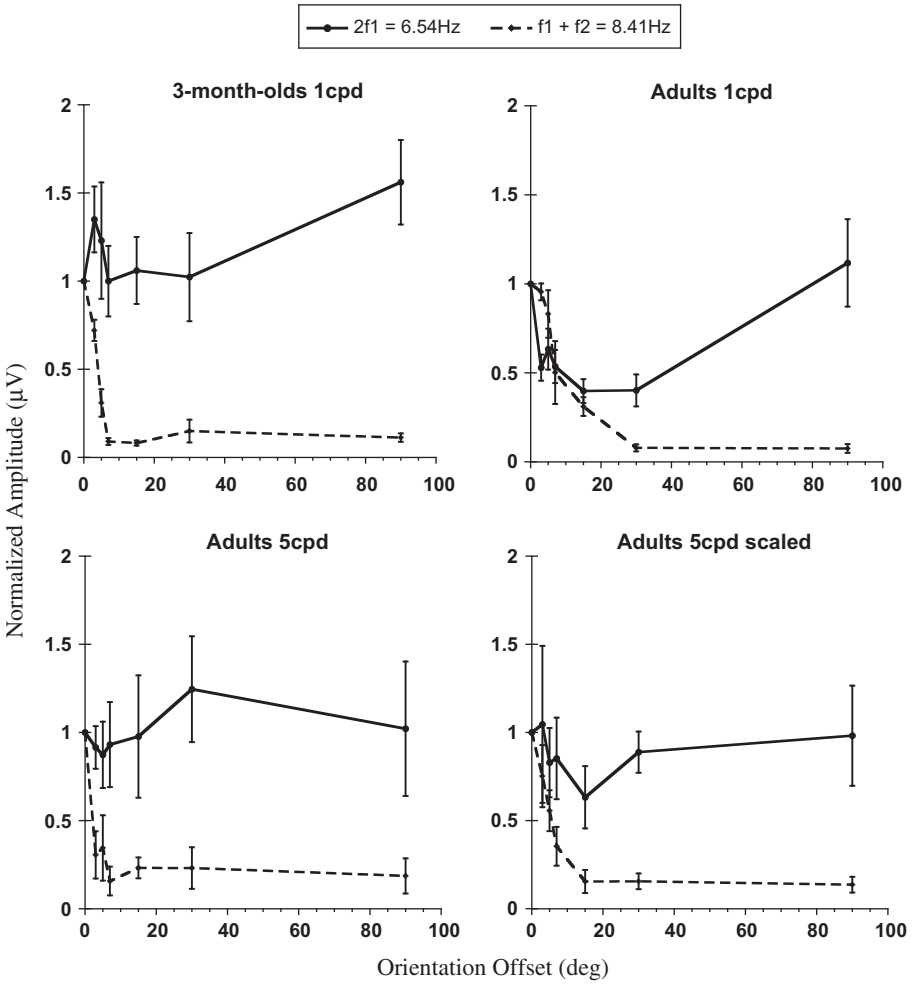

Fig. 2. Mean normalized VEP responses at the 2f1 (solid lines) and sum intermodulation (dashed lines) frequencies (bars are *SE*) for 3-month-olds, Adults 1 cpd, Adults 5 cpd, and Adults 5 cpd scaled conditions. The data demonstrate the orientation tuning of the sum intermodulation term.

Table 2
Shows the main effects and post-hoc comparisons for the 2f1 ANOVA.

2f1 ANOVA	Group	Offset	Group × Offset
Main effects:	$F(3, 163) = 2.31$, $p < 0.01$	$F(6, 163) = 1.67$, $p = 0.13$, *ns*	$F(18, 163) = 0.830$, $p = 0.66$, *ns*
Post-hoc for main effect of group			
Group	Significant differences		
Infants ($M = 1.18$, $SE = \pm 0.07$)	Adults 1 cpd ($p < 0.001$) ($M = 0.66$, $SE = \pm 0.08$)	Adults 5 cpd scaled ($p = 0.049$) ($M = 0.89$, $SE = \pm 0.08$)	
Adults 1 cpd ($M = 0.66$, $SE = \pm 0.08$)	Adults 5 cpd ($p < 0.01$) ($M = 1.00$, $SE = \pm 0.08$)		

Table 3
Shows the main effects and post-hoc comparisons for the 2f2 ANOVA.

2f2 ANOVA	Group	Offset	Group × Offset
Main effects:	$F(3, 163 = 4.80$, $p < 0.005$	$F(6, 163) = 1.33$, $p = 0.24$, *ns*	$F(18, 163) = 0.42$, $p = 0.98$, *ns*
Post-hoc for main effect of group			
Group	Significant differences		
Infants ($M = 1.26$, $SE = \pm 0.15$)	Adults 5 cpd scaled ($p < 0.05$) ($M = 1.91$, $SE = \pm 0.19$)		
Adults 1 cpd ($M = 0.99$, $SE = \pm 0.19$)	Adults 5 cpd scaled ($p < 0.01$) ($M = 1.91$, $SE = \pm 0.19$)		
Adults 5 cpd ($M = 1.10$, $SE = \pm 0.19$)	Adults 5 cpd scaled ($p < 0.05$) ($M = 1.91$, $SE = \pm 0.19$)		

in the current study, potentially as a result of the normalization process in the analysis.

2.2.2. Intermodulation tuning

The third component (and most important for determining the VEP tuning bandwidths) of the spectral analysis was the second-order sum IM term (8.41 Hz), which is indicative of a nonlinear interaction between the inputs to a common nonlinear mechanism driven by both stimuli (Regan & Regan, 1987). A two-way analysis of variance (ANOVA) for the sum frequency was performed with orientation offset (0°, 3°, 5°, 7°, 15°, 30°, 90°) as the within subjects factor and group (Infants 1 cpd, Adults 1 cpd, Adults 5 cpd, and Adults 5 cpd scaled) as the between subjects factor. See Table 4 for the main effects and post-hoc comparisons for group and orientation offset. These results are indicative of orientation tuning of this IM term. A series of one-way ANOVA's were also performed for each group, with orientation offset as the dependent variable, to explore the group by orientation-offset interaction, (indicating differences in the orientation tuning between the four groups) (see Table 5).

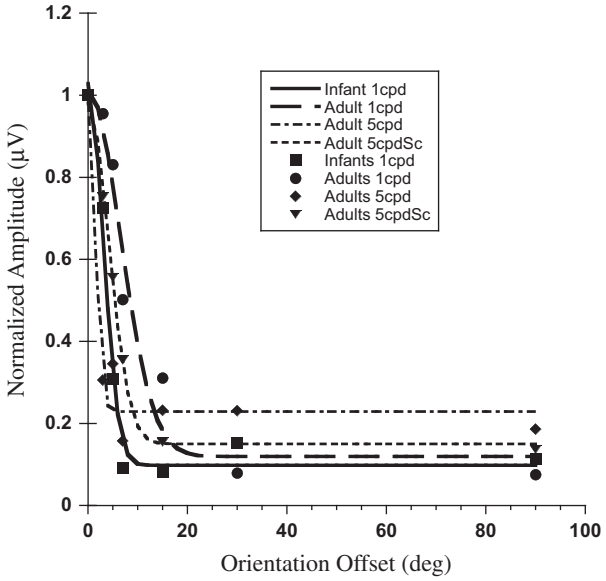

Fig. 3. Normalized sum intermodulation amplitudes averaged across participants, with the function fitted to these data. Error bars have been omitted for clarity.

Table 4
The sum intermodulation term ANOVA.

Intermodulation Tuning	Group	Offset	Group × Offset (see Table 5)
Main effects:	$F(3, 163) = 9.85$, $p < 0.01$	$F(6, 163) = 80.33$, $p = 0.001$	$F(18, 163) = 3.84$, $p < 0.001$
Post-hoc tests for group and orientation offsets			
Group means	Sig. differences for group	Orientation means	Sig. differences for orientation
Infants ($M = 0.35$, $SE = \pm0.02$)	Adults 1 cpd, $p < 0.05$	0° ($M = 1.00$, $SE = \pm0$)	p all <0.005
Adults 1 cpd ($M = 0.54$, $SE = \pm0.02$)	Adults 5 cpd $p < 0.05$ ($M = 0.35$, $SE = \pm0.02$)	3° ($M = 0.68$, $SE = \pm0.04$)	Greater than all larger angles ($p < 0.005$)
		5° ($M = 0.51$, $SE = \pm0.04$)	Greater than all larger angles ($p < 0.005$)
		7° ($M = 0.28$, $SE = \pm0.04$)	Greater than 30° and 90° offsets ($p < 0.05$)
		15° ($M = 0.20$, $SE = \pm0.04$)	15°, 30°, and 90° were no different from each other ($p > 0.05$)
		30° ($M = 0.15$, $SE = \pm0.04$)	
		90° ($M = 0.13$, $SE = \pm0.04$)	

2.2.3. VEP tuning bandwidths

The VEP tuning function for the sum IM term was estimated by fitting individual subjects' normalized amplitude function with a Gaussian as follows:

$$Amp = R_{\min} + R^* e^{-\theta^2/2\sigma^2}$$

where *Amp* is the normalized response amplitude, $R_{\min}$ is the response minimum or baseline, R is the maximum response, θ is the orientation offset and σ is the width of the function.

The sigma parameter was used as an estimate of the VEP bandwidth. The infant functions were only fit if they included four or more data points, leading to 11 of 15 infant data sets being included in this analysis. The median σ values for the different conditions were: 2.98° for infant 1 cpd, 4.69° for adult 1 cpd, 1.19° for adult 5 cpd, and 3.32° for adult scaled 5 cpd, corresponding to half-width, half-height values of 3.5°, 5.5°, 1.4° and 3.9°, respectively. The Wilcoxon rank-sum test indicated that the 1 cpd infant and adult data had significantly different medians ($z = 3.015$, $p = 0.0026$), whereas the infant and adult 5 cpd ($z = -1.809$, $p = 0.0704$) or scaled 5 cpd ($z = 0.503$, $p = 0.6153$) did not. The σ values for fits to the normalized data averaged across subjects, shown in Fig. 3 were: 3.00° for infant 1 cpd, 6.50° for adult 1 cpd, 1.42° for adult 5 cpd, and 4.12° for adult scaled 5 cpd, corresponding to half-width, half-height values of 3.5°, 7.7°, 1.7° and 4.8° respectively.

Table 5
The Group by Offset interaction for the sum intermodulation term. A one-way ANOVA was performed for each Group. The significance relative to the baseline (floor) amplitude is indicated in the post-hoc testing.

Infants	Adults 1 cpd	Adults 5 cpd	Adults 5 cpd scaled
$F(6, 58) = 92.08$, $p < 0.001$	$F(6, 35) = 20.17$, $p < 0.001$	$F(6, 35) = 13.99$, $p < 0.001$	$F(6, 35) = 12.35$, $p < 0.001$
Post-hoc tests revealing full VEP tuning bandwidths			
Amplitude reached baseline by 7° offset ($M = 0.09$, $SE = \pm0.02$) as it did not differ from larger offsets ($p > 0.05$)	Amplitude reached baseline by 15° offset ($M = 0.31$; $SE = \pm0.13$) as it did not differ from larger offsets ($p > 0.05$)	Amplitude reached baseline by 3° offset ($M = 0.31$; $SE = \pm0.09$) as it did not differ from larger offsets ($p > 0.05$)	Amplitude reached baseline by 5° offset ($M = 0.55$, $SE = \pm0.12$) as it did not differ from larger offsets ($p > 0.05$)

3. Experiment II

The VEP orientation-tuning bandwidths measured in the first experiment are much narrower than values typically reported for single-units in either cat (Chen, Dan, & Li, 2005; Hubel & Wiesel, 1965) or monkeys (DeValois et al., 1982; Gur et al., 2005; Xing et al., 2005). The degree of orientation tuning estimated from the VEP may differ from that measured with single-units for a number of reasons, including differences in the stimuli and response measures used, the sampling biases of the two techniques and the effects of anesthesia. Recent physiological studies (Chen et al., 2005; Gur et al., 2005; Mazer, Vinje, McDermott, Schiller, & Gallant, 2002; Ringach, Hawken, & Shapley, 2003; Xing et al., 2005) have also suggested that single neuron orientation tuning is dependent upon the size of the stimuli. For example Xing et al. reported that in the anesthetized macaque that tuned suppression is greater with larger stimuli and likely comes from outside the classic receptive field (CRF). Orientation bandwidths of neurons in the anesthetized cat have been reported to be 59.3° full width at half-height when measured with stimuli that are restricted to the classical receptive field but decrease 13.6–20.5% when the stimulus is enlarged to two to four times the CRF, respectively (Chen et al., 2005). Okamoto, Naito, Sadakane, Osaki, and Sato (2009) also working in the anesthetized cat, found narrower bandwidths for spatially extended stimuli compared to those restricted to the classical receptive field (approximately 20° versus 25° FWHM, respectively). Given this, we would expect that our VEP bandwidths measured with extended stimuli might be narrower than those measured with spatially restricted stimuli, such as those typically used in single-unit studies. In order to more directly compare our VEP tuning bandwidths to those reported in the single-cell literature, we therefore re-measured the VEP orientation bandwidth using small stimuli that more closely resemble those used in previous studies in previous single-unit studies of cat and monkey.

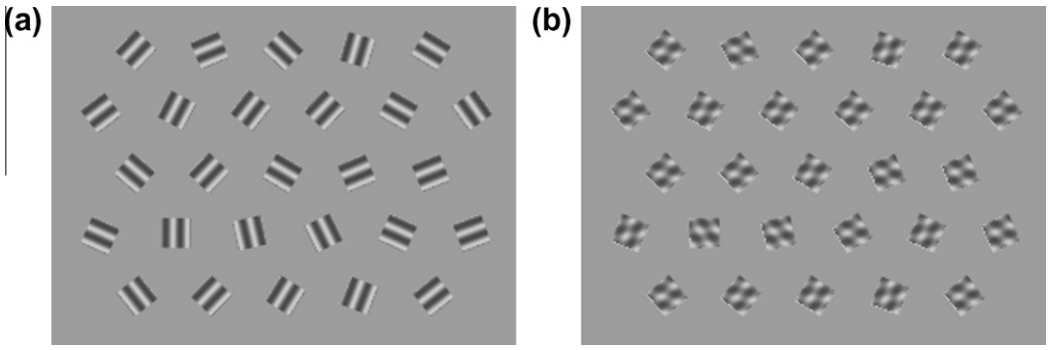

Fig. 4. Frames taken from the Experiment 2 stimuli at 0° offset (a) and 30° offset (b). The test stimulus (which counterphase-reversed at 3.14 Hz) and the mask stimulus (which reversed at 5.27 Hz) were superimposed in interlaced frames, with the test remaining at a constant orientation while the mask was rotated to the appropriate orientation offset. All patches were randomly offset relative to each other.

3.1. Methods

3.1.1. Participants

Six adults (two of whom participated in Experiment 1) with corrected to normal vision participated in this experiment (six females; one male). They ranged in age from 19 to 42 years (M = 29.68, SD = ±10.23). Informed consent in accordance with the Indiana University IRB was obtained from each of these participants.

3.1.2. Stimuli

The same display and recording system as described for Experiment 1 was used. Participants viewed 27 square stimulus patches consisting of two overlapping and truncated spatial sinusoids, each of 40% Michelson contrast, that were presented on interlaced raster lines. The patches subtended 2° (or approximately two grating cycles at 1 cpd), with a mean luminance of 104 cd/m^2. To reduce long-range interactions between the patches, the 27 squares were presented at random baseline orientations (see Fig. 4). The test remained at that baseline orientation and was counterphase-reversed at 3.27 Hz (f1). The second spatial sinusoid (the mask) was presented at orientation offsets relative to the test of 0°, 7°, 15°, 30°, 45°, and 90° and was counterphase-reversed at 5.14 Hz (f2).

3.2. Results

The new data are shown in Fig. 5, which shows response tuning as a function of orientation offset for the small patch stimuli as squares. Comparable data for the large patch stimuli of the first experiment are shown as circles. In each case, the response amplitudes were normalized to the 0° offset values. The solid and dashed lines show fits to the tuning data as described in the first experiment. Using the same bandwidth measure as in Experiment 1, we found the median σ value for the adults in Experiment 2 was 10.29°, corresponding to a half-width, half-height value of 12.04°, and the mean σ value to be 9.66°, corresponding to 11.3° half-width, half-height. These values are greater than those obtained from adults in Experiment 1 and are closer to the values found in prior single cell data, although still somewhat narrower.

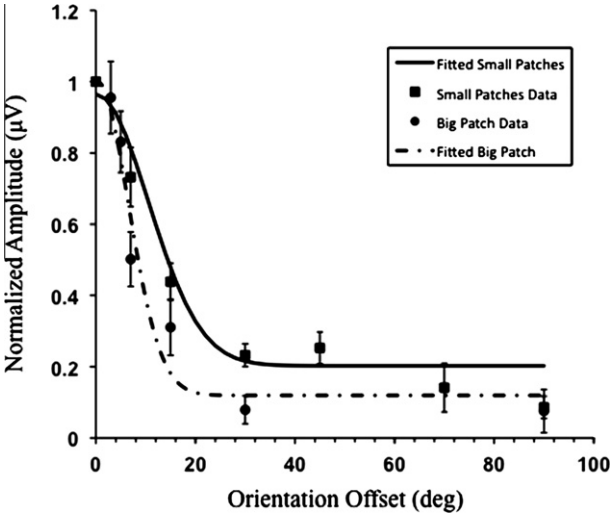

Fig. 5. Comparison of tuning functions for extended grating and small patch stimuli. VEP tuning bandwidth at half-height increased from 5.5° to 12.04° as stimulus size decreased. The lines are the fitted functions and the points are the normalized empirical data. Error bars are SEM of the empirical data.

4. General discussion

We have conducted an analysis of cortical orientation tuning in typically developing 3-month-old infants and adults. Our tuning estimate was based on a nonlinear component of the VEP that indicates the degree to which individual neurons jointly process stimuli with varying degrees of orientation offset (Regan & Regan, 1988). Using this measure, we found the nonlinear interaction between the two inputs (test and mask) decreased with increasing offset. Infants had a median half-width, half-height bandwidth of 3.5° for extended grating stimuli. Adults in the 1 cpd condition had a median half-width, half-height of 5.5°, with values of 1.4° and 3.9° for the 5 cpd and 5 cpd scaled conditions, respectively. These are the first measurements in infants made with this technique and they indicate that the infant intermodulation is strongly orientation tuned. Further, the results from Experiment 1 also demonstrated that there was no release from masking with increased orientation offset for either the 2f1 or 2f2 frequencies. This finding is consistent with Candy et al. (2001), whose highest contrast equaled that used in this study.

The VEP bandwidths from Experiment 1 are much narrower than typical estimates of single cell tuning (e.g. Chen et al., 2005; DeValois et al., 1982; Gur et al., 2005; Xing et al., 2005). We used a masking technique for quantifying orientation bandwidth from the VEP (Regan & Regan, 1987) whereas the single-unit literature has used single stimuli. VEP amplitude depends only weakly on the orientation of a single stimulus (Arakawa et al., 2000) and reflects biases in the *distribution* of the underlying single-neuron tunings. The intermodulation measure, on the other hand presumably reflects the degree to which individual

neurons jointly process the two oriented inputs in our displays. Single neuron tuning has not been measured in this way and the value of orientation bandwidth measured in this way may differ from that measured with single stimuli. Quantitative comparison of our VEP tuning bandwidths and those of single-units is made difficult because several factors determine the degree of orientation tuning that is measured. In the single-unit literature, the degree of orientation tuning depends on cell type, with simple cells showing narrower tuning than complex cells (Gur et al., 2005; Schiller et al., 1976), on the layer of cortex in which the cell is recorded (Gur et al., 2005) and on whether anesthesia is used (Gur et al., 2005). It is not clear at this point whether the VEP samples preferentially from cells that are more or less orientation tuned. Stimulus size is also an important factor in determining the absolute value of orientation tuning of single-units: the addition of stimulation of the non-classical receptive field (Chen et al., 2005; Gur et al., 2005; Ringach et al., 2003) decreased orientation bandwidth. In Experiment 2, we measured wider VEP orientation bandwidths with stimuli that are more comparable in size to the classical receptive field – a trend that is consistent with the results of several recent single-unit studies (Chen et al., 2005; Gur et al., 2005; Ringach et al., 2003; Xing et al., 2005). Our estimate of 12° FWHM bandwidth with small stimuli is closer the range of tunings of the most selective cells in the alert macaque (e.g. median = 11.5° in Gur et al., 2005). It should also be noted that in Experiment 1, the 5 cpd scaled stimulus yielded broader bandwidths than the (larger) 5 cpd stimulus, which is again consistent with the effect noted in the single-unit studies.

4.1. Consideration of possible artifacts that might lead to spuriously narrow tuning in the VEP

Before concluding that the intermodulation response directly reflects the orientation tuning of individual neurons, we consider a possible alternative source of intermodulation that is not orientation selective but that nonetheless could generate a (spurious) orientation tuning. Because the stimuli used to drive the response were spatially superimposed, it is possible that a simple intensity non-linearity could generate this sum frequency response (e.g. in a retinal photoreceptor or ganglion or geniculate population that is not orientation tuned, but is sampling the two temporal frequencies at the same spatial location (Regan & Regan, 1987)). It seems unlikely that this is the case, however, for the following reason. If the IM response at the electrode were the sum of the responses at each location in the image in this case, one would predict that there would only be significant IM in the aligned condition and not in the offset conditions. There are two stimulus states at any one time in the aligned condition (as shown in the zero degree offset panel in Fig. 1) and four stimulus states in the other offset conditions (as shown in the other panels in Fig. 1). The intermodulation in the aligned condition would be expected to be in phase in the two states, and therefore combine to generate an intermodulation response at the electrode. In the offset conditions the additional two stimulus states would be in phase with each other, but 180° out of phase with the first pair. Thus the intermodulation response would indeed be expected to reduce with orientation offset between the two stimuli if the intermodulation resulted from the sum of untuned local intensity nonlinearities, but a relatively simple quantitative prediction can be made by considering the proportion of the stimulus in the different intermodulation phases as a function of orientation offset. For a 12° patch, 1 cpd stimulus in the aligned condition it would be 100% in phase. For a 3° offset it would be 42% in one phase and 58% in a phase 180° shifted. This difference of 16% becomes zero by the 30° offset. Thus the resulting summed intermodulation at the electrode would be expected to drop quickly from the aligned condition and change little from 3° to 90° of relative offset. Neither the adult nor infant 1 cpd data demonstrate this pattern. The 12° patch, 5 cpd stimulus would have a 3% difference in area at the two phases by 3° of offset and is zero by 10° of offset, while the 5 cpd scaled stimulus reaches 3% difference by 5° of offset. These predictions are not quantitatively consistent with the data shown in Fig. 2.

The results are consistent, however, with the general properties of linear receptive fields, followed by an accelerating output nonlinearity, (Movshon, Thompson, & Tolhurst, 1978) and more generally divisive gain control models where the output of such receptive fields is normalized by a broad pool of other neurons (Candy et al., 2001; Heeger, 1992; Heeger & Adelson, 1989). The VEP technique does not reveal the specific location of nonlinear processes giving rise to the intermodulation response in the EEG, but the fact that the VEP bandwidths are narrow despite averaging across the population of neurons contributing to the EEG suggests that the tuning of these processes is quite robust. The fact that the intermodulation is present in the second-order sum also suggests that the response is generated at a stage of processing that still has access to the linear first harmonic inputs, potentially implying an early stage of processing, prior to any cascade of significant nonlinear processes. There are few measurements of single-cell orientation tuning that have used simultaneously presented test and mask stimuli (see Bonds, 1989) and these did not measure the intermodulation component that we find to be strongly tuned. Direct comparison of field potential and spike-discharge tuning functions using the temporal tagging technique and the intermodulation measure would be of great value in determining the quantitative relationship between our measure of orientation bandwidth and that obtained from single-unit recordings.

4.2. Receptive field scaling

Experiment 1 not only examined orientation tuning, but also explored different comparisons between the adults and infants (1 cpd, 5 cpd, and 5 cpd scaled). The goal was to include different developmental models (Banks & Crowell, 1993; Wilson, 1988) that have been proposed for receptive field maturation. The fovea is not mature at 3-months of age and there is significant postnatal migration of photoreceptors (Yuodelis & Hendrickson, 1986). The 12° stimulus used in Experiment 1 would include the fovea and other more peripheral regions, which in combination, may not be well represented by any one of these models. Interestingly, there were no significant differences collapsed across orientations between infants and adults in the 5 cpd condition for 2f1, 2f2 and the sum intermodulation frequencies, nor were there significant differences in terms of the median σ values for infants and adults in both the 5 cpd and 5 cpd scaled conditions. These results demonstrate, however, that none of the adult datasets are dramatically different from the infant data, when compared with immaturities in many aspects of spatial vision at 3 months of age.

4.3. Comparison with previous studies

Thus far, much of what we know about the development of orientation tuning has come from studying monkeys, (e.g. Hubel & Wiesel, 1968; Wiesel & Hubel, 1974), cats and ferrets (see Chapman, Godecke, and Bonhoeffer (1999) for a review). As mentioned earlier, Wiesel and Hubel (1974) found that the orientation tuning of cortical receptive fields is quite mature in macaques whose eyes were sutured shut at birth. Wiesel and Hubel therefore reported that, at least for monkeys, early visual experience is not necessary for basic development of the neural framework for orientation mechanisms. They hypothesized that the maturity of

orientation-tuned structures in macaques is genetically programmed (see also Hubel & Wiesel, 1963; Horton & Hawking, 1996).

The human infant visual system is immature at birth (Atkinson & Braddick, 1979; Atkinson et al., 1988) and much development occurs postnatally (Johnson, 1990). Burkhalter et al. (1993) examined the development of the human infant visual cortex, specifically in terms of vertical and horizontal connections. The horizontal connections linking neurons with similar orientation preferences (Gilbert & Wiesel, 1989) were only starting to emerge around 4-months of age (Burkhalter et al., 1993). While these connections may be necessary for linking information across space (Bosking, Zhang, Schofield, & Fitzpatrick, 1997) their immaturity does not seem to impact dramatically the tuning of neurons responding to the overlapping stimuli in the present study. The current VEP data suggest that even though the horizontal connections are not developed in 3-month-old infants, local (overlapping) orientation tuning appears to be within a factor of two of adult-like.

Past behavioral studies have demonstrated immaturities in infants' abilities to discriminate orientation (Atkinson et al., 1988; Bornstein et al., 1986) and distinguish contour shapes (Baker, Tse, Gerhardstein, & Adler, 2008; Gerhardstein, Kovacs, Ditre, & Feher, 2004). Given those results, one may hypothesize that orientation tuning in young infants would be broader than found in adults. As mentioned above, the current study suggests that tuning, as represented by the bandwidth of the sum intermodulation term, is well developed at 3-months-of age, to within a factor of two of adult values. Given that the tuning estimates varied with the size of the stimulus in adults, it is not possible to draw a clear conclusion about the most likely model of development, but it is notable that the infant data fall within a factor of two of the bandwidths found in all three adult conditions. Presumably this relative maturity could underlie their developing sensitivity to more complex cues such as disparity (e.g. Braddick, 1996) and vernier offset (e.g. Brown, 1997).

Although the infants' orientation tuning is relatively mature, this does not necessarily mean that all aspects of their orientation processing are adult-like (see Candy et al., 2001). For example, it has been reported (using human cadavers) that long-range horizontal connections within layers 2/3 do not reach full maturity until around 15-months of age (Burkhalter et al., 1993). Therefore, in the context of the infant behavioral literature on contour integration (Baker et al., 2008; Gerhardstein et al., 2004) the present data suggest that there may be significant development of orientation selective mechanisms responsible for long-range interactions across space (see Hou, Pettet, Sampath, Candy, & Norcia, 2003). In conclusion, though basic orientation tuning may be somewhat adult-like at 3-months of age, additional maturation of higher order orientation processing is likely to occur.

Acknowledgments

Thanks to: Diane Goss, Danielle Teel, Shrikant Bharadwaj, Kyle Gilbert, and Sylvia Mishoulam for help with scheduling and testing participants. Also thanks to David Regan for comments on a poster version of these data.

Ruth L. Kirschstein National Research Service Award, Grant Number: HD 07475.

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